

TAXONOMY OF THE TURBINATE SHAPED SEED CONE
TAXA OF *JUNIPERUS*, SECTION *SABINA*: SEQUENCE
ANALYSIS OF nrDNA AND FOUR cpDNA REGIONS

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ABSTRACT

An analysis of the turbinate shaped seed cone taxa of *Juniperus* sect. *Sabina* is presented, based on nrDNA and four cpDNA regions. These DNA data revealed several previously unknown affinities. Plants collected as *J. przewalskii* f. *pendula* were found to be *J. convallium* (***J. convallium forma pendula*, comb. nov.**). Several infraspecific taxa were found to be so distinct to warrant recognition at the specific level: *J. fargesii* (= *J. squamata* var. *fargesii*); ***J. uncinata*, stat. & comb. nov.** (= *J. recurva* var. *uncinata*); *J. carinata* (= *J. pingii* var. *carinata*); ***J. rushforthiana*, stat. nov.** (= *J. indica* var. *rushforthiana*). *J. squamata* f. *wilsonii* is best treated as ***J. squamata* var. *wilsonii*, comb. nov.** *Phytologia* 94(3): 388-403 (December 1, 2012).

KEY WORDS: Taxonomy, *Juniperus*, section *Sabina*, turbinate seed cones, nrDNA, petN-psbM, trnS-trnG, trnD-trnT, trnL-trnF, *J. convallium* f. *pendula*, comb. nov., *J. uncinata*, stat. & comb. nov., *J. squamata* var. *wilsonii*, com. nov., *J. rushforthiana*, stat. nov.

The turbinate shaped seed cone junipers of section *Sabina* comprise a distinct clade (Mao et al., 2010), having seed cones with elongated, pointed tips (Fig. 1). The cone elongation is more

apparent in immature cones. Although most of the taxa have only scale-like leaves in the adult foliage (Fig. 1), several taxa have only decurrent type leaves in the adult foliage (Fig. 2). Decurrent leaves (juvenile) have a sheath that clasps the stem and a free blade that may be appressed (*J. squamata* var. *squamata*, Fig. 2), free at about 30° - 45° (*J. sq. f. wilsonii*), free at about 60° - 90° (*J. sq. var. fargesii*), elongated and slightly free (10° - 20°) in *J. morrisonicola*, to very appressed as in *J. recurva* and *J. coxii* (Fig. 2). Because the seed cones are similar, decurrent leaf shape has been used to lump *J. squamata*, *J. sq. f. wilsonii* and *J. sq. var. fargesii* and *J. morrisonicola* (*J. sq. var. morrisonicola* (Hayata) H-L. Li & H. Keng).

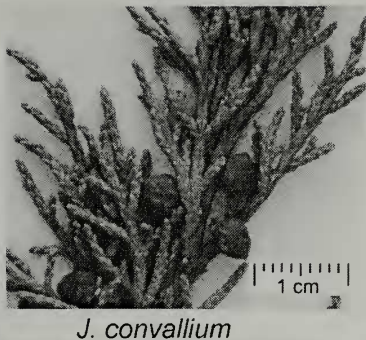


Figure 1. *J. convallium* leaves and turbinate shaped seed cones.

Juniperus squamata has, historically, included several taxa with decurrent leaves that don't fit into other turbinate junipers. Part of the confusion seems to have arisen by the nature of the type specimen from a sheet that has three specimens from at least two and likely, three taxa (Fig. 3); Farjon (2005, p. 382) designated this sheet as the lectotype for *J. squamata*, but he failed to indicate which one of the specimens. As the sheet contains three plants, the lectotype is clarified here as: *W. S. Webb W 6043C* (lectotype K-W, right-most specimen on sheet *W 6043C*). The original description of Buch.-Ham. [Lambert, Descr. Pinus 2: 17 (1824)] reads "leaves 3, appressed, imbricate, ovate-oblong, acute, acuminate; withered persistent; inflexed when young with apex somewhat obtuse, fruits ovate and umbilicate at the top, branches and branchlets crowded, terete, stem prostrate." Clearly, the right-most specimen best fits the description of *J. squamata*.

A comparison of leaves from the type specimens of *J. squamata*, f. *wilsonii* and var. *fargesii* (Fig. 4) reveals the nature of the appressed leaf blades in *J. squamata* and f. *wilsonii*, and the free, very

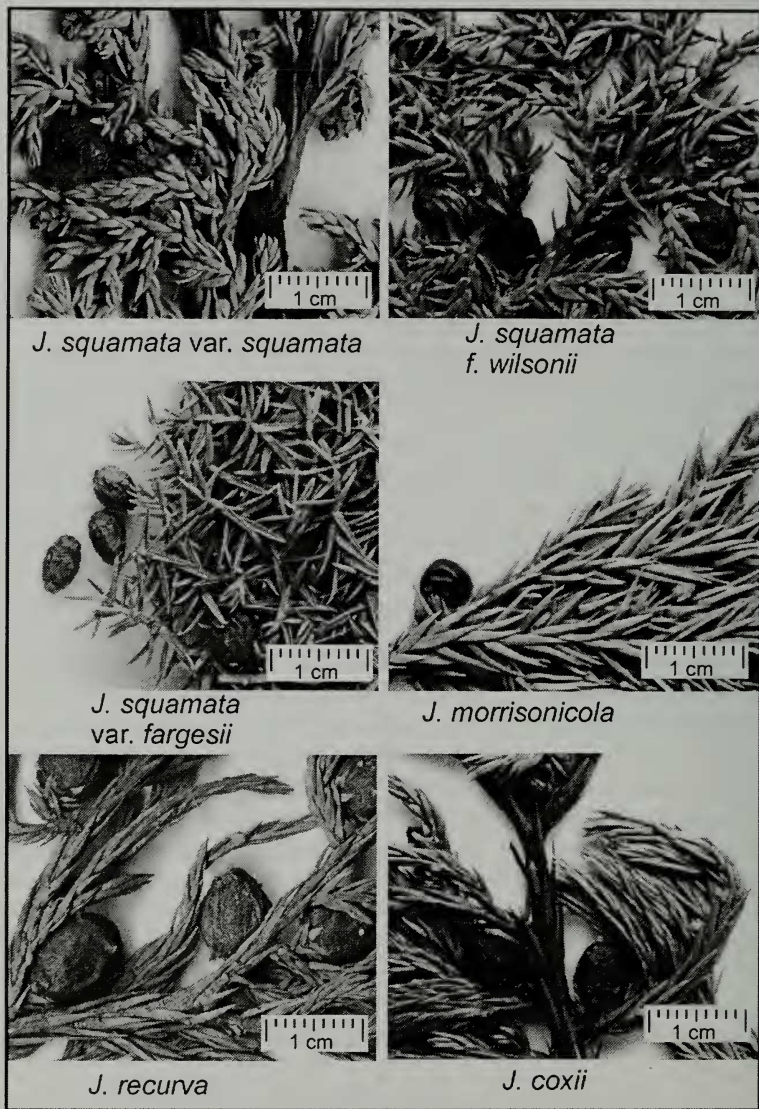


Figure 2. Comparison of the decurrent leaves of six turbinate seed cone taxa.



Figure 3. Photo of type of *J. squamata* Buch.-Ham., W. S. Webb, W 6043C at K (K000395285). Left-most: cf. *J. indica*, center: cf. *J. squamata* var. *fargesii*, right-most: *J. squamata*.

divergent leaf blades in *J. squamata* var. *fargesii*. The seed cones of *J. squamata* appear larger than those of *J. s. f. wilsonii* (Fig. 4).

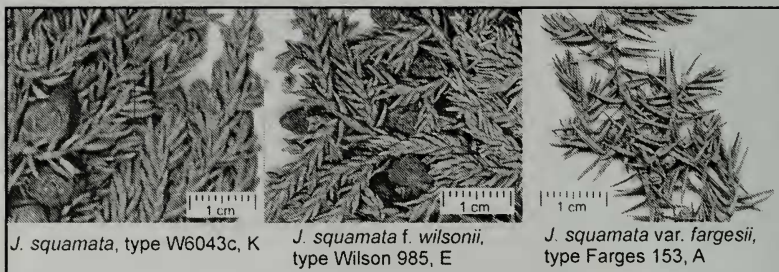


Figure 4. Comparison of leaves from the type specimens of *J. squamata*, *f. wilsonii* and var. *fargesii*.

Although Mao et al. (2010) analyzed several of the turbinate junipers (*J. convallium*, *J. coxii*, *J. indica*, *J. komarovii*, *J. pingii*, *J. przewalskii*, *J. pseudosabina*, *J. saltuaria*, *J. squamata*, *J. tibetica*), they did not include *J. indica* var. *caespitosa*, *J. i.* var. *rushforthiana*, *J. microsperma*, *J. morrisonicola*, *J. pingii* var. *carinata*, *J. przewalskii* f. *pendula*, *J. recurva* var. *recurva*, *J. r.* var. *uncinata*, *J. squamata* var. *fargesii* and *J. s. f. wilsonii*.

The purpose of the current study is to present analysis of all the turbinate seed cone taxa of section *Sabina* using the most informative nuclear (nrDNA - ITS) and cpDNA regions (petN-psbM, trnS-trnG, trnD-trnT, trnL-trnF).

Table 1. Comparison of Adams and Farjon taxonomic treatments of taxa in this study. Taxa with DNA sequencing support for a modified taxonomic status are in bold.

Adams(2011)	Farjon (2005, 2010)	Supported, this study
<i>J. convallium</i>	<i>J. convallium</i>	<i>J. convallium</i>
<i>J. coxii</i>	<i>J. recurva</i> v. <i>coxii</i>	<i>J. coxii</i>
<i>J. indica</i>	<i>J. indica</i>	<i>J. indica</i>
<i>J. i. var. caespitosa</i>	<i>J. i. var. caespitosa</i>	<i>J. recurva</i> var. <i>caespitosa</i> ?
<i>J. i. var. rushforthiana</i>	<i>J. indica</i>	<i>J. rushforthiana</i>
<i>J. komarovii</i>	<i>J. komarovii</i>	<i>J. convallium</i> var. <i>komarovii</i> ?
<i>J. microsperma</i>	<i>J. convallium</i> var. <i>microsperma</i>	<i>J. microsperma</i>
<i>J. morrisonicola</i>	<i>J. squamata</i>	<i>J. morrisonicola</i>
<i>J. pingii</i>	<i>J. pingii</i>	<i>J. pingii</i>
<i>J. p. var. carinata</i>	<i>J. p. var. wilsonii</i>	<i>J. carinata</i>
<i>J. przewalskii</i>	<i>J. przewalskii</i>	<i>J. przewalskii</i>
<i>J. p. f. pendula</i>	<i>J. przewalskii</i>	<i>J. convallium</i> f. <i>pendula</i>
<i>J. pseudosabina</i>	<i>J. pseudosabina</i>	<i>J. pseudosabina</i>
<i>J. recurva</i>	<i>J. recurva</i>	<i>J. recurva</i>
<i>J. r. var. uncinata</i>	<i>J. recurva</i> ?	<i>J. uncinata</i>
<i>J. saltuaria</i>	<i>J. saltuaria</i>	<i>J. convallium</i> var. <i>saltuaria</i> ?
<i>J. squamata</i>	<i>J. squamata</i>	<i>J. squamata</i>
<i>J. s. var. fargesii</i>	<i>J. squamata</i>	<i>J. fargesii</i>
<i>J. s. f. wilsonii</i>	<i>J. pingii</i> f. <i>wilsonii</i>	<i>J. s. var. wilsonii</i>
<i>J. tibetica</i>	<i>J. tibetica</i>	<i>J. tibetica</i>

MATERIALS AND METHODS

Specimens used in this study: *J. convallium*, Adams 6781-83, 17 km e Tewo, Gansu, China, *J. communis* L. var. *communis*, Adams 7846, 7847, Stockholm, Sweden (outgroup), *J. coxii*, Adams 8137-38, clone from Type tree, Abbotsmarsh Arboretum, UK, ex Burma, Chimili valley, *J. indica*, Adams 8775-77, Dumpa, Jomson, Nepal, *J. indica* var. *caespitosa*, Adams 7625-27, near Yangjin Gompa, Nepal, *J. indica* var. *rushforthiana*, Adams 8140-41, Abbotsmarsh Arboretum, UK, ex Lingshi, Bhutan, *J. komarovii*, Adams 8518-20, near Zhe Gu Mtn., Maerkan County, Sichuan, China, *J. microsperma*, Adams 8522-24, near Zhe Gu Mtn., Maerkan County, Sichuan, China, *J. morrisonicola*, Adams 8681-2, Younger Bot. Gard., Scotland, ex Taiwan, *J. pingii*, Adams 8506-7, near White Horse Mtn., Deqin County, Yunnan, China, *J. pingii* var. *carinata*, Adams 8497-99, near White Horse Mtn., Deqin County, Yunnan, China, *J. pseudosabina*, Adams 7808-10, 30 km n Jarkent (Paniflor), Kazakhstan, *J. przewalskii*, Adams 6775-77, 25 km w Jone, Gansu, China, *J. przewalskii* f. *pendula*, Adams 6779, Langmusi, Gansu, China, *J. recurva*, Adams 7215, 7217, 7219, Cholan Pati lodge, Nepal, *J. recurva* var. *uncinata*, Adams 7212-14, Lauri Binayak, Nepal, *J. saltuaria*, Adams 6789-91, on Duoer River, 23 km e Forestry Station, Gansu, China, *J. squamata*, Adams 6795-96, Xian Bot. Garden, Shaanxi, China, *J. s.* var. *fargesii*, Adams 8491-93, near White Horse Mtn., Zhongdian County, Yunnan, China, *J. s.* f. *wilsonii*, Adams 5521, Accession 1010-64A, cultivated from seeds from *E. H. Wilson* 985 (Holotype) collection, Arnold Arboretum, USA, ex. China, *J. tibetica*, Adams 8512-16, on Maerkan River, near Zhe Gu Mtn., Maerkan County, Sichuan, China. Voucher specimens are deposited in the herbarium, BAYLU, Baylor University.

DNA extraction, PCR amplification, sequencing and data analyses - see Adams and Schwarzbach (2012a).

RESULTS AND DISCUSSION

The turbinate seed cone junipers are relatively uniform with a few distinct groups (Fig. 5). As previously reported (Adams 2011), *J. recurva* and *J. indica* taxa are in a clade, but well resolved from *J.*

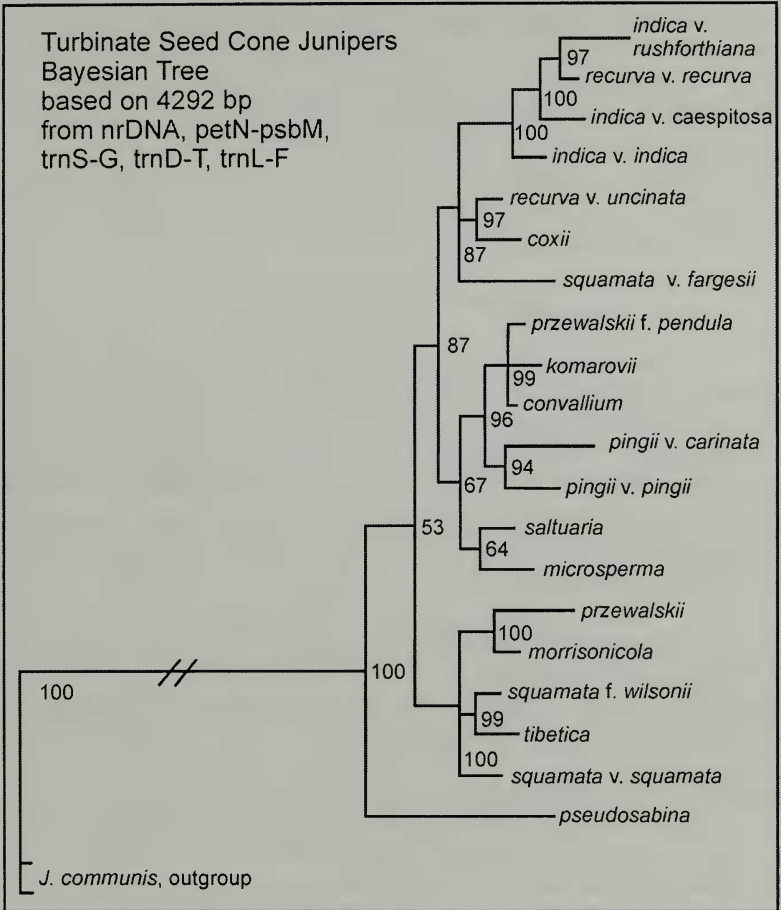


Figure 5. Bayesian tree for the turbinate seed cone taxa, sect. *Sabina*. Numbers at the branch points are posterior probabilities (as percent).

indica var. *rushforthiana* (Fig. 5). *Juniperus recurva* is a small tree with lax foliage and tightly appressed decurrent leaves (Fig. 2), and *J. indica* has generally erect foliage with typical scale-like leaves of (cf. Fig. 1), otherwise, the taxa are similar in morphology (Adams 2011). *Juniperus recurva* var. *uncinata* is in a clade with *J. coxii* and *J. squamata* var. *fargesii*.

Juniperus convallium, *J. komarovii* and the putative *J. przewalskii* f. *pendula* are in an unresolved clade (Fig. 5). *J. pingii* and *J. p.* var. *carinata* are in a clade, but are well differentiated. *J. microsperma* and *J. saltuaria* are associated with the *J. convallium* - *komarovii* clade (Fig. 5). Finally, it is noteworthy that *J. przewalskii*, *J. morrisonicola*, *J. squamata* f. *wilsonii*, *J. s.* var. *squamata* and *J. tibetica* form a distinct clade (Fig. 5). *J. pseudosabina* is well supported as an outlier to the other turbinate junipers (Fig. 5).

Taxonomic Considerations

In addition to illuminating phylogeny, DNA sequence data are useful in validating and elucidating taxonomy. Coding all nucleotide substitutions and indels (as single mutations) resulted in 225 mutational events (MEs). These 225 MEs were used to construct a minimum spanning network (Fig. 6). Two patterns are immediately visible: the central nature of *J. convallium* among the taxa, and relative uniformly large differences between taxa (even taxa in the same species, such as *J. indica*, *J. i.* var. *caespitosa*, *J. i.* var. *rushforthiana*, Fig. 6).

The most obvious taxonomic anomaly is the difference of only 2 MEs (out of 225) between *J. convallium* and putative *J. przewalskii* f. *pendula* (Fig. 6). Clearly, this pendulous form is a part of *J. convallium* and should be recognized as:

Juniperus convallium forma *pendula* (Cheng & L-K. Fu) R. P.

Adams, **comb. nov.** **Basionym:** *Sabina przewalskii* (Kom.) W-C., Cheng & L-K. Fu f. *pendula* Cheng & L-K. Fu, Acta Phytotax. Sin. 13(4): 86. 1975. Type: PE. *Juniperus przewalskii* f. *pendula* R. P. Adams and G-L. Chu, J. Essential Oil Res. 6:17. 1994.

The *J. convallium* - *komarovii* - *saltuaria* complex is separated by only 6 - 8 MEs (Fig. 6). Adams and Schwarzbach (2012a, 2012b) found varieties in *Juniperus* sections *Juniperus* and *Sabina* to differ by 6 - 8 MEs, whereas generally accepted species differed by 10 - 12 to 20 MEs (using the same set of gene regions). Both Adams (2011) and Farjon (2005, 2010) recognize these three as species in their monographic treatments (Table 1); it seems wise to continue such

recognition , although there is only marginal support for such treatment in the DNA data set.

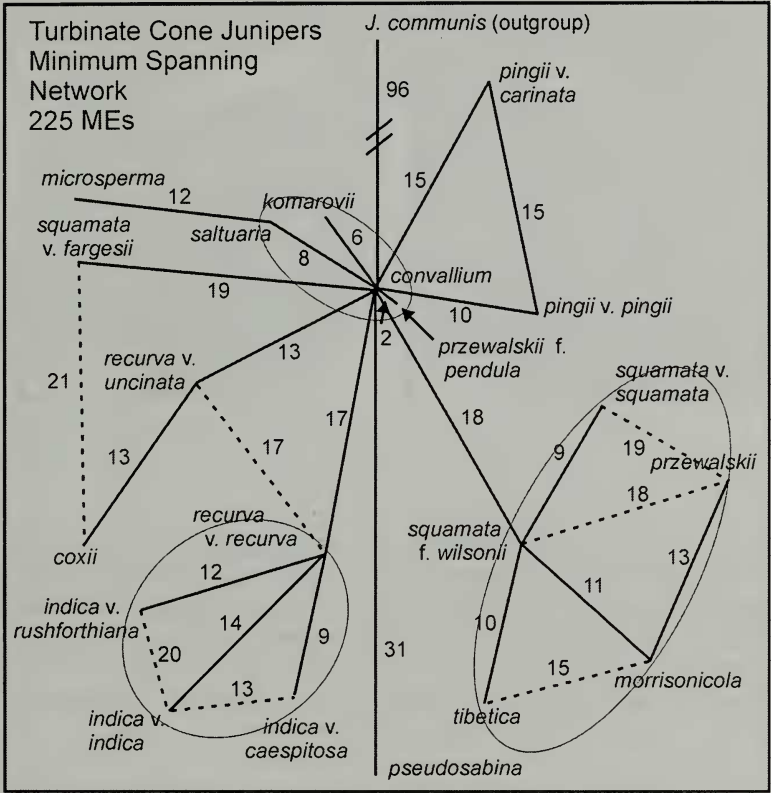


Figure 6. Minimum spanning network based on 225 MEs from nrDNA, and cpDNA (petN-psbM, trnS-G, trnD-T, trnL-F). Numbers next to the lines are the number of MEs separating taxa. Dashed lines are the second nearest link.

Juniperus pingii is a tall tree, with long, sharp-pointed decurrent leaves and *J. pingii* var. *carinata* is a small shrub with short, scale-like leaves (Fig. 7). *Juniperus pingii* differs by only 10 MEs

from *J. convallium* (Fig. 6), whereas *J. p.* var. *carinata* differs by 15 MEs from both *J. pingii* and *J. convallium* (Fig. 6). The taxa are easy to identify by their leaves and habit. The morphology and DNA data support the recognition of *J. carinata* R. P. Adams, [Biochem. Syst. Ecol. 28: 541 (2000)].

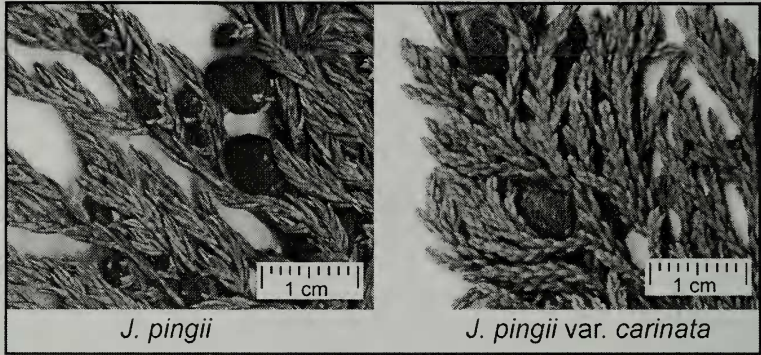


Figure 7. Leaves and seed cones of *J. pingii* and *J. p.* var. *carinata*.

Juniperus squamata var. *fargesii* has very unusual leaves with the blades divergent at 60 - 90° from the stem (Fig. 4) these differing from *J. squamata*. The placement of *J. s.* var. *fargesii*, in both the Bayesian tree and the network, is distantly separated from *J. squamata* (Figs. 5, 6). It is 19 MEs from *J. convallium* and 21 MEs from *J. coxii* (Fig. 6). Both morphology and DNA support the recognition of *Juniperus fargesii* (Rehder & E. H. Wilson) Kom.

Juniperus recurva var. *uncinata* is a shrub with hooked branchlets (Adams et al. 2009) from Nepal and appears, morphologically, to be closely related to *J. recurva* (Fig. 8). *Juniperus recurva* and *J. r.* var. *uncinata* hybridize in the area of Cholan Pati, Nepal (Adams et al. 2009). However in the present analysis, the taxa are in different clades (Fig. 5). *Juniperus r.* var. *uncinata* differs by 13 MEs from both *J. convallium* and *J. coxii*, and by 17 MEs from *J. recurva* (Fig. 6). These differences give strong support for the recognition of:

Juniperus uncinata (R. P. Adams) R. P. Adams, stat. & comb. nov.,
Basionym: *Juniperus recurva* var. *uncinata* R. P. Adams, Phytologia
 91(3): 365 (2009). Type: BAYLU.



Figure 8. Comparison of the leaves and seed cones of *J. recurva* and *J. uncinata*. Note the hooked branchlets of *J. uncinata*.

Juniperus squamata f. *wilsonii* was collected by E. H. Wilson on his 1907-1909 China expedition. Seeds from this collection were germinated by Arnold Arboretum and three plants are currently in cultivation at Arnold Arboretum; DNA from one of these was utilized in the present study. The blade tips are more spreading in cultivation than in the type specimen (Fig. 9). *Juniperus* s. f. *wilsonii* is loosely associated with *J. squamata* (Fig. 6) by 9 MEs vs. 10 MEs to *J. tibetica* and 11 MEs to *J. morrisonicola*). The category *forma* is used to recognize a minor variant in a population that differs by only one or a few genes. In short, DNA data indicate that *J. s. f. wilsonii* is well differentiated from *J. squamata* (Fig. 6) and supports its recognition as a variety:

Juniperus squamata var. *wilsonii* (Rehder) R. P. Adams, comb. nov.
Basionym: *Juniperus squamata* f. *wilsonii* Rehder, J. Arn. Arb. 1: 191
 (1920). Type: A.

Sabina pingii (W. C. Cheng ex Ferre) W. C. Cheng & W. T. Wang
 var. *wilsonii* (Rehder) W.C. Cheng & L.K. Fu, Fl. Reipubl. Pop.
 Sin. 7: 356 (1978)

- S. squamata* (Buch.-Ham. ex D. Don) Antoine var. *wilsonii* (Rehder) W. C. Cheng & L.K. Fu, [Chin. title; see Fl. Sichuan 2:177 (1983)] 1: 320 (1972)
- S. wilsonii* (Rehder) W.C. Cheng & L.K. Fu, Forest Sci. Techn. 4: 455 (1981)
- J. wallichiana* Hook. f. & Thomson ex E. Brandis var. *loderi* Hornibr., Gard. Chron., ser. 3, 85: 50 (1929)
- J. squamata* Buch.-Ham. ex D. Don var. *loderi* (Hornibr.) Hornibr. in Chittenden, Rep. Cult. Conif.: 74 (1932)

Clearly, *J. s.* var. *wilsonii* is not part of *J. pingii* (Figs. 3, 4, Table 1) and the present DNA data give no support for the recognition of *J. pingii* f. *wilsonii* of Silba (1984).

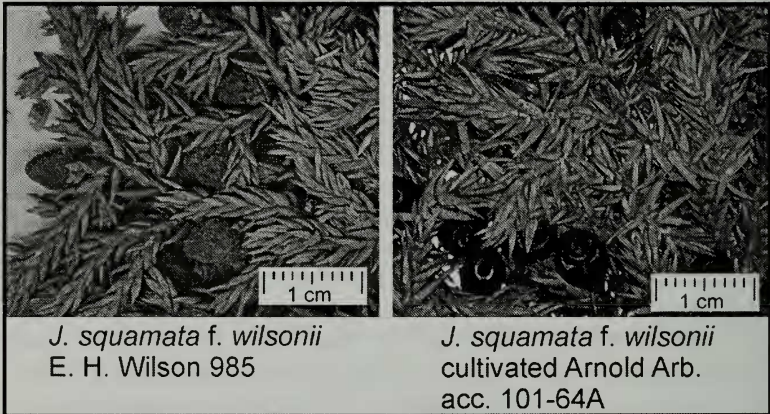


Figure 9. Comparison of *J. s.* f. *wilsonii*, specimen and cultivated.

It should be noted that the recognition of *J. s.* var. *wilsonii*, although well supported by the present DNA data, does not resolve the taxonomy of the *J. squamata* - *tibetica* - *morrisonicola* - *przewalskii* complex (Fig. 6). This group differs by 18 MEs from *J. convallium* (Fig. 6) with internal links of 9 to 13 MEs. At present, it seems prudent to continue to recognize these four species (Table 1).

The *J. indica* - *recurva* group differs by 17 MEs from *J. convallium* and from 9 to 14 MEs within the group (Fig. 6). *Juniperus*

indica var. *rushforthiana* differs by 12 MEs from *J. recurva* and 20 MEs from *J. indica* (Fig. 6) giving support for recognition as a species:

***Juniperus rushforthiana* (R. P. Adams) R. P. Adams, stat. nov.**

Basionym: *Juniperus indica* var. *rushforthiana* R. P. Adams, *Phytologia* 90(2): 244 (2008). Type: BAYLU.

Juniperus indica var. *caespitosa* differs by 13 MEs from *J. indica* and 9 MEs from *J. recurva*. Additional sampling and sequencing is in progress to better understand this group.

A graphic summary of the current turbinate juniper taxonomy is shown in Figure 8.

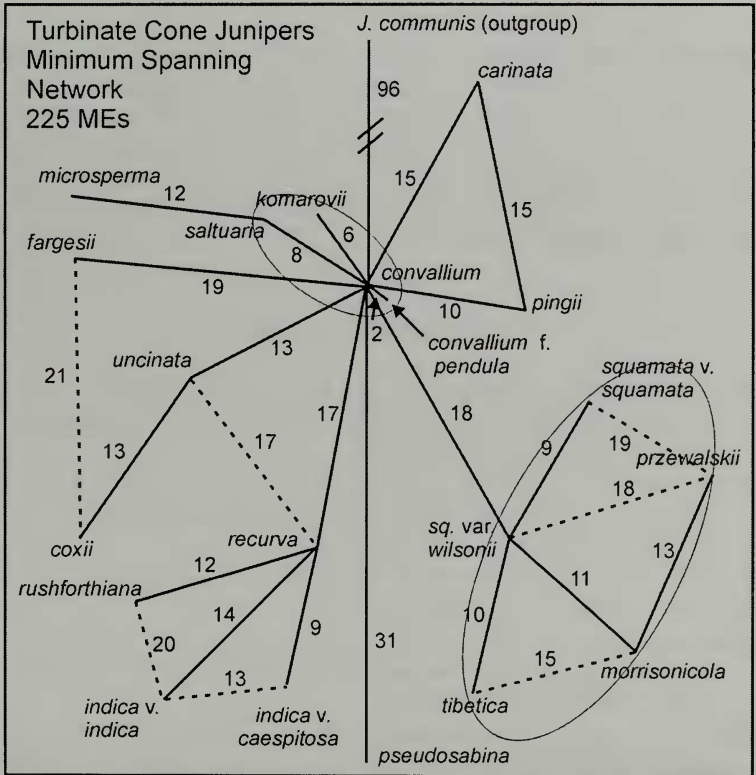


Figure 10. Minimum spanning network of the turbinate junipers as recognized in this study.

The turbinate junipers are found chiefly in western China and the eastern Himalayan Mtns. in small isolated populations that appear to be relictual. Clearing of land for agriculture, grazing by goats, use of foliage for incense (Nepal), and fuel wood utilization have decimated many of these species' populations. Whether these extremely small populations (often only a few individuals) are representative of the original taxa is not known, but genetic drift is very likely responsible for the large variation in morphology encountered. Additional studies involving extensive field work are needed in the turbinate junipers.

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